A NEW GENE IN THE SECOND CHROMOSOME OF DROSOPHILA AND SOME CONSIDERATIONS ON DIFFERENTIAL VIABILITY.

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Morgan and Lynch (BIOL. BULL., '12) and Morgan (*Science*, '12) have reported the linkage relations of two non-sex-linked genes, black and vestigial.[1] Morgan considered these two genes as lying in a "second chromosome," the first chromosome being the sex chromosome. He showed that in the female there was a considerable amount of crossing over between these two genes, but in the male there was none at all so far as the data showed.

At the time when this linkage between black and vestigial was first observed we were engaged in a systematic search for linkage between non-sex-linked genes in *Drosophila*. One of us (Bridges) had already observed in the F_2 generation of a cross of black by curved[2] that no black curved flies appeared. We interpreted this case as one of linkage of such a strong order that no crossing over had taken place. On the basis of this linkage we concluded that curved was in the same chromosome as black, that is, in a "second chromosome." The similar work of Morgan on black and vestigial showed that the non-appearance of the double recessive in a case in which two second chromosome recessives entered the F_1 from opposite parents, could be explained on the basis of lack of crossing over in the male. The present paper shows that the same explanation applies to the case of black by curved, and further deals with the determination of the amount of crossing over in the female between the black and the curved loci.

An individual heterozygous for two allelomorphic pairs as *AB*, *ab* may form four kinds of gametes, namely *AB*, *ab*, *Ab*, and

[1] Vestigial was at that time called wingless.

[2] "Curved" a wing mutant discovered by Bridges is characterized by the thin texture of the wings which are held out widely from the body and curved. This and other mutants are shortly to be described in detail by Morgan and Bridges.

aB. In case of linkage, however, the two new combinations (here *Ab* and *aB*) are not represented in as large numbers in the gametes as are the original combinations (*AB* and *ab*). The actual ratio in which these gametes are formed can be calculated only very indirectly from an F_2 zygotic ratio, and if the two F_1 individuals are forming the gametes in different ratios, the calculation may be impossible.

As suggested by Baur (*Verh. naturf. Ver. Brünn*, '11) what is needed is to test these double heterozygotes by an individual all of whose gametes are of one kind, namely, recessive for both factors in question (*aa*, *bb*) since in this case they will not mask any combination in the gametes tested. Baur's method, then, is to test double heterozygotes by double recessives. The proportion in which the zygotes appear is a direct measure of the gametic proportions. In such zygotic proportions there are two equal classes representing original combinations (in the example *AB* and *ab*) and two other equal classes representing recombinations or crossovers. In calculating the percentage of crossing over therefore, we add these cross over classes together and divide by the total number of zygotes. Since in the case of sex-linked characters the male producing sperm is analogous to a double recessive, the F_2 males will in effect always be such a back cross test.

Flies with curved wings from pure stocks were mated to stock black flies. The F_1 flies were gray not-curved, that is, like the wild fly in appearance. F_2 consisted of:

	Wild Type.	Black.	Curved.	Black Curved.
	391	194	168	0
	458	226	165	0
Total	849	420	333	0

The absence of black curved in F_2 is the result to be expected if there is no crossing over in the male, no matter what the percentage of crossing over may be in the female. However, if there is crossing over in the female a few of the F_2 blacks should be heterozygous for curved and a few of the curved heterozygous for black. The most advantageous procedure to get the double recessive from these F_2 flies is to mate in mass cultures the blacks

of one sex to the curved of the other sex. In F_3 if there has been crossing over in the F_1 ♀ there should appear some of the double recessives or some single recessives heterozygous for the other single recessive. In fact some blacks appeared which gave, in F_4, 3 black : 1 black curved. From these black curved individuals a pure stock of the double recessive was obtained directly.

That the absence of black curved flies in F_2 was really due to lack of crossing over in the male was shown by making "back-cross" tests of doubly heterozygous males as follows:

Black × curved.
↓
F_1 ♂♂ × black curved ♀♀.
↓

Wild Type.	Black.	Curved.	Black Curved.
0	35	40	0
0	44	31	0
0	25	20	0
0	18	15	0
0	45	60	0
0	39	33	0
0	90	83	0
0	50	51	0
0	29	34	0
0	375	367	0

From the converse cross of wild by black curved described below, two tests of F_1 males were made, with the following results:

Wild × black curved.
↓
F_1 ♂♂ × black curved ♀♀.
↓

Wild Type.	Black.	Curved.	Black Curved.
108	0	0	88
71	0	0	57
179	0	0	145

No new combinations of characters (crossovers) appeared in the 1,066 flies from the two converse experiments. This is in exact agreement with Morgan's results on black vestigial, and is somewhat more significant, since, as will appear below, there

is in our case more crossing over in the females than in the combination studied by Morgan.

We have seen that when black and curved enter F_1 separately, one class, namely, the double recessive, does not appear in F_2, for the reason that a double recessive gamete is not formed in the F_1 male through lack of crossing over. But if black and curved enter from the same parent then half the gametes of the F_1 male are doubly recessive, and, therefore, give in a back cross test the amount of crossing over in the female. The other half of the gametes of the male are the double dominant class and in consequence half of the flies fall into a single double dominant class. If, then, there is crossing over in the female in the ratio of one crossover gamete to n of the original combination, the F_2 will consist of $n : 1 : 1 : n$ zygotes from the doubly recessive sperm, and a like total, that is $2n + 2$, from the doubly dominant half of the sperm. The F_2 proportions expected are therefore $3n + 2 : 1 : 1 : n$ and the ratio of each single recessive class (1) to the double recessive class (n) gives the gametic ratio directly. The results of such a cross as that described appear below:

Wild × black curved.

↓

F_2

	Wild Type.	Black.	Curved.	Black Curved.
	298	27	15	48
	88	7	5	8
*s**[1]	252	21	15	56
*s**	310	19	28	79
	948	74	63	191

The occurrence of black and of curved flies in this experiment demonstrates that crossing over takes place, and from the evidence of the preceding experiments, we conclude that it must have been in the females. That this is the correct interpretation is shown by direct tests of such females. As stated above, the percentage of crossing over can be calculated directly from tests

[1] In all counts reported here *s* signifies that the record includes only the offspring of a single female. For reasons which will appear in future publications from this laboratory it has seemed advisable to include only the first cultures obtained from any females. When later cultures are available but have been omitted, the record will be marked with an asterisk.

of this nature. In the following table the calculation for each culture and for the total is given.

Wild × black curved
↓
F_1 ♀ × black curved ♂♂.
↓

	Wild Type.	Black.	Curved.	Black Curved.	Per Cent. of Crossovers.
	96	31	21	96	21.3
s*	63	20	17	78	20.7
s*	103	34	40	102	26.5
s*	106	27	44	105	25.2
s*	112	34	55	127	27.2
s	130	38	49	144	24.1
	610	184	226	652	24.5

Similar tests of F_1 females from the converse cross of black × curved gave:

	Wild Type.	Black.	Curved.	Black Curved.	Per Cent. of Crossovers.
	88	203	212	80	28.8
	68	161	159	80	31.6
	56	221	256	69	20.8
	67	373	286	84	18.7
	70	252	240	64	21.4
s	17	97	95	26	18.3
s	27	113	92	21	19.0
s	33	152	141	28	17.2
s	18	100	99	27	18.4
s	11	50	56	8	15.2
s*	45	144	150	55	25.4
s*	41	134	119	52	27.1
s*	56	157	148	40	23.9
s*	47	135	95	29	24.8
	644	2,292	2,148	663	22.7

Adding the figures from these two converse experiments gives 1,717 crossovers in 7,419 flies, or 23.1 per cent. of crossovers. Because of double crossing over, which is known from unreported experiments to occur within this distance, 23.1 is slightly less than the actual chromosomal distance apart of these two loci.[1]

It will be seen from the two above tables that there is apparently a rather wide range of variability in the percentage of crossing over in different cultures. This variability is much greater than one would expect to find if it were due entirely to

[1] See Sturtevant (*Jour. Exp. Zool.*, '13) for a discussion of linear series of genes within a chromosome.

chance deviations; and one might therefore be led to suppose that it is due to an actual variation in the strength of linkage. While this conclusion may be correct, it is not necessary, since there is another important factor which must be considered—namely, the effects of differential viability. In order to get definite data regarding the manner of action of this disturbing element we have made some crosses in which it may be studied without the complication of linkage. If curved flies heterozygous for black ($B\ c_v\ b\ c_v$) be mated to blacks heterozygous for curved ($b\ C_v\ b\ c_v$), the same four classes of flies as in the above tables should be produced, but now in equal numbers. If equality is not shown the deviation cannot be due to linkage, but must probably be attributed either to the error of random sampling, or to differential viability. The results actually obtained in these experiments are shown in the following table:

Black[1] (het. for c_v) × curved (het. for b).

	Wild Type.	Black.	Curved.	Black Curved.
I.*s*.	31	31	22	29
II.	46	48	40	48
III.	35	27	30	14
IV.*s*.	44	53	33	65
V.*s*.	14	31	18	22
VI.	52	50	56	41
VII.	47	31	24	36
VIII.*s*.	43	31	2	20
IX.*s*.	13	7	10	15
X.*s*.	37	43	43	28
XI.*s*.	37	51	36	26
XII.*s*.	14	14	14	17
XIII.*s*.	76	78	56	46
	489	495	384	407

That there really is no linkage present is evident from the totals, since the complementary classes give totals of 879 and 896, respectively. The deviation of these numbers from equality is less than half the standard error—a very close approximation to expectation. These totals show some effects of differential viability, especially in that the curved flies were less numerous than those with normal wings. A study of the individual bottles brings out some other interesting points. It is obvious that

[1] The first five cultures below were from curve ♀ × black ♂, the others from the reciprocal cross (black ♀ × curved ♂).

viability may not always produce the same kind of effect. For instance, in culture VIII., the curved class ran far behind all three others, yet in VI. it was the largest class of all. The same relations are shown by the black curved class in XIII. and IV. respectively, and in several other cases. It also appears that the viability difference between two classes differing in two characters is not always merely a summation of the effects produced by these characters separately. Thus in culture VII., since the curved and the black class are each behind the normal class, we might expect the class which is both black and curved to be still further behind—yet it is really ahead of both single recessive classes. In culture VIII. black is slightly behind normal, but black curved is far ahead of curved (gray). It is obvious from these considerations that it is not possible to work out "coefficients of viability" and use them for making corrections in our data, since with respect to viability the deviations are not constant in amount or direction. However, it is to be noted that when conditions are made as favorable as possible the error from viability is reduced considerably, and often becomes very slight indeed. There is evidence which indicates that differential viability is often due to unequal sensitiveness to starvation, dryness, or similar unfavorable conditions. Several of the cultures recorded in the last table above were purposely kept under various poor conditions (small bottles, little food, etc.,) in order to test this point. Cultures II., III., and VIII. are examples showing the results produced, and III. and VIII. are among the most aberrant cultures in the table. The remedy, then, would seem to be in choosing mutants which are of nearly the same vigor as the normal, and in keeping the cultures in good condition—plenty of room and good food.

Even under these conditions there may be a high mortality, but that this need not always be a *differential* mortality is indicated by an experiment which we have carried out. Three females from the cross of black by curved were tested by backcrossing to black curved males. The eggs were counted daily, and the offspring produced were recorded, with the following result:

Wild Type.	Black.	Curved.	Black Curved.	Total.	Total No. Eggs.
34	91	96	32	253	550

Thus although less than half the eggs produced flies, there is no evidence of differential variability, since the complementary classes are approximately equal, as expected.

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